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# ANTIBACTERIAL NATURE OF DRAGLINE SILK OF *Nephila pilipes* (Fabricius, 1793)

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## ABSTRACT

Spiders silk is a high performance natural fiber with diverse biological functions. In this study, native dragline silk of *Nephila pilipes* was tested for its ability to inhibit the growth of some bacteria. Inhibition of bacteria was observed after 24 hours. Dragline silk of *Nephila pilipes* could inhibit the growth of *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa.* The inhibition was observed even after one year old silk. Thus, dragline silk of *Nephila pilipes* could be a potential source as antibacterial agent.
**Key words:** *Nephila pilipes*, dragline silk, Antibacterial.

## INTRODUCTION

For many decades, scientists have been fascinated with the favorable mechanical properties of spider silk. Orb web spider *Nephila pilipes* produces six different types of silks like dragline, auxiliary spiral, sticky capture silk, attachment discs, egg sac silk, swathing bands etc. Each type of silk is used for different purposes by the spider. It uses dragline silk for creating web framework and also as safety line which can be used as emergency line to escape from enemies.

In nature, birds line their nests internally with spider silk. This observation made us to think and therefore, we tried to investigate the antimicrobial activity of *fresh* silk of *Nephila pilipes.*

## MATERIALS AND METHODS

Dragline silk samples of *Nephila pilipes* were collected from the woods of Melghat. Antibacterial activity of dragline silk of *Nephila pilipes* was tested for *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae*.

Antimicrobial assay was performed using agar plates. Nutrient broth or liquid nutrient medium devoid of agar was prepared for cultivation of desired bacterial strain.

**Composition of nutrient broth medium**:- Peptone – 5.0 g; Beef extract – 3.0 g; Sterilized distilled water – 1000 ml; pH – 7.

10 ml of nutrient liquid broth was taken in test tube. 2-3 identical colonies were picked up from bacterial culture plate and transferred to the broth. The tube was incubated for bacteria to grow. Above procedure was followed to prepare liquid broth of *E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae.*

Nutrient or culture media were prepared in laboratory by weighing and dispensing the individual ingredients.

**Composition of nutrient agar:** Peptone – 5.0 g; Beef extract – 3.0 g; Sterilized distilled water – 1000 m Agar agar powder –20.0 g; pH – 7

The nutrient agar was poured into petri plate and allowed to solidify for about 3 hours. A cotton swab was dipped in the inoculums and suspension was swabbed over the entire surface of agar to give a lawn. Dragline silk threads (~ 1 mg) were first gently washed in sterilized distilled water and placed on the surface of inoculated agar. This process was carried out in flame zone of burner in a laminar air flow to avoid any kind of contamination. Plates were incubated for 24 hours at 37 $^{0}$C and examined to observe zone of inhibition.

## OBSERVATIONS AND RESULTS

Antibacterial activity of dragline silk was studied after 24 hours of incubation. Fig- 1 shows the results of antimicrobial activity of silk for different bacteria. After 24 hours of incubation, a clear zone of inhibition appeared around the dragline silk sample of *Nephila pilipes* for *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. These bacteria could not grow around the silk threads and formed a zone of inhibition around silk. Diameter of clearing zones to the nearest millimeter were measured (Table- 1).

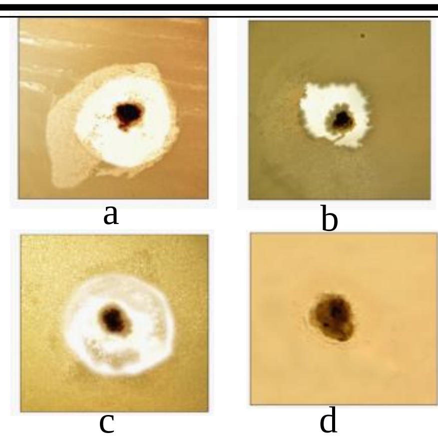


**Fig.-1: Antibacterial activity of dragline silk of *Nephila pilipes* in the lawn of-**
a. *Escherichia coli*
b. *Staphylococcus aureus*
c. *Pseudomonas aeruginosa*
d. *Klebsiella pneumoniae*

Inhibition zone was not observed around dragline silk samples in the lawn of *Klebsiella pneumoniae*. This strain of bacteria grew up to the margin of silk but

not on silk threads (Fig.-1d). Dragline silk showed stronger inhibition on *Pseudomonas aeruginosa.* Little bacterial growth was observed around the silk and tiny colonies in the clearing zone. The antibacterial property was observed in one year old silk also.

**Table- 1:** Antibacterial activity of dragline silk of *Nephila pilipes* after 24 hours of incubation.

| Bacteria tested | Diameter of inhibition zone (in mm) |
|---|---|
| *Escherichia coli* | 13 |
| *Staphylococcus aureus* | 11 |
| *Pseudomonas aeruginosa* | 14 |
| *Klebsiella pneumoniae* | —— |

## DISCUSSION

Testing of antimicrobial activity of *Nephila pilipes* silk gave strong evidence supporting the view that spider silk has antibacterial properties. The results are given in Fig- 1.

However, agar plates showed growth of number of tiny colonies in the clearing zone. A possible explanation for this is that there might be some bacteria already present on the silk, which might have grown on nutrient agar. These bacteria might be from the dust particles stuck to silk. Few of them might have survived.

It is possible that spider webs that are on or close to the ground would be likely to possess more antimicrobial compounds because of the high occurrence of microbes on the ground (Stotzky, 1997). Conversely, it would be expected that orb web spiders, which builds their webs off the ground, would less likely to possess antimicrobial properties due to their webs coming into less contact with microbes (Wright, 2011). But, many a times bacteria like *Staphylococcus*, *Pseudomonas* and *Escherichia coli* exist in air and dust (Griffin, 2007). There are number of chances that bacteria like *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* may be present in the forest soil. This could explain why *Nephila* silk had an inhibitory effect on these bacteria, but not on *Klebsiella pneumoniae*. Numbers of *Nephila pilipes* webs are found on roadside in the forest. Although *Nephila pilipes* build their orb webs above the ground, the dust particles from soil may stick up to silk threads due to blowing air and frequent movements of cattle and other animals in the forest.

Agar plates showed no bacterial growth on the silk threads. This antimicrobial property might have evolved in silk in order to resist microbial decomposition. Reduced decomposition might be advantageous if it decreases the energetic cost of web maintenance and/or the level of harmful microbes to which spider is exposed

(Wright and Goodacre, 2012). Such situation is beneficial for the orb weaver like *Nephila pilipes* which do not construct web daily, rather their webs are long lasting.

*Nephila pilipes* generally sit at the hub of web, which is made up of dragline or ampullate silk. So, another benefit of this silk being antimicrobial is that this spider gets protected from microbes which may have adverse effect on the health of spider.

One year old dragline silk samples were also used to test antimicrobial activity in the present investigation and they exhibited antimicrobial effect like that of fresh samples. This indicates that the antimicrobial properties are maintained for several days, probably, more than one year.

Before inoculation on agar plates, silk threads of *Nephila pilipes* were gently washed in distilled water. In nature also, spider webs are frequently subject to rain and being wetted by rain. So, it is expected that antimicrobial properties of this spider silk threads would be maintained in wetted silk also.

## CONCLUSION

Dragline silk of *Nephila pilipes* inhibits the growth of bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*, which is the common cause of wound infections. So, it may be used as an excellent material of bandage for wound dressing. Above mentioned bacteria are also important pathogens in nasocomial infections. So, this silk can be recommended for preparation of clinical masks used in hospitals. This high quality bio-material may used for manufacturing antibacterial cloths for children/patients in hospitals and also for manufacture of surgical threads.

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